

## R4150 Test Report

## Experiment 1: Validation of RNA Extraction Performance of R4150 for Conventional /Polysaccharide /Polyphenolic Plant Samples

- Sample type: 100mg plant leaves
- Elution volume: 100µl
- Extraction time: 20 minutes (single sample)
- Test kit: R4150
- Detection method: nanodrop and agarose gel electrophoresis

## Experimental data:

Sample	Sample	Sample	Antioxidant	RNA Conc.	Yield	A260/280	A260/230
Туре		Amount		(ng∕µl)	₽g		
Ordinary polyphenol plant	Banyan leaves I	100mg	Mercaptoethanol	155	15.58	2.09	1.79
			TCEP	177	17.77	2.13	1.84
	Mango leaves <b>I</b>	100mg	Mercaptoethanol	364	36.42	2.19	1.23
			TCEP	453	45.35	2.11	2.1
	Eucalyptus leaves III	100mg	Mercaptoethanol	296	29.58	2.16	1.32
			TCEP	299	29.95	2.11	1.96
Difficult-to- extract polyphenol plant	Tea	100mg	Mercaptoethanol	121	12.13	1.79	1.17
	leaves <b>IV</b>		TCEP	120	12.21	1.89	1.66
	Loquat leaves <b>V</b>	100mg	Mercaptoethanol	141	14.14	2.10	1.33
			TCEP	155	15.52	2.12	2.1
	Grape leaves <b>VI</b>	100mg	Mercaptoethanol	211	21.17	2.11	1.31
			TCEP	227	22.74	2.19	2.05
Polysaccha ride plant	Rose	100mg	Mercaptoethanol	233	23.34	2.10	1.30
	leaves <b>VII</b>		TCEP	267	26.73	2.19	2.11
	Sweet potato leaves	100mg	Mercaptoethanol	530	53.5	2.12	2.41
	VIII		TCEP	510	51.5	2.03	2.12
	Paper mulberry leaves <b>IX</b>	100mg	Mercaptoethanol	426	42.67	2.09	1.41
			TCEP	439	43.91	2.19	1.87
Ordinary plant	Pepper leaves <b>X</b>	100mg	Mercaptoethanol	285	28.58	2.18	1.28
			TCEP	314	31.47	2.11	2.06
	Tomato leaves <b>XI</b>	100mg	Mercaptoethanol	307	30.76	2.11	1.48
			TCEP	296	29.64	2.12	1.84







## Conclusion: [A universal odorless plant RNA extraction kit]

The difficult-to-extract polyphenol samples, easy-to-extract polyphenol samples, polysaccharide samples, and conventional plant leaves were used as samples in this experiment. After grinding into small powders using liquid nitrogen, they were extracted using the <u>HiPure Plant RNA Plus Kit (R4150)</u>. At the same time, the extraction were compared by the different addition of 5% mercaptoethanol and 20µm TCEP (non-toxic and odorless mercaptoethanol substitute). The sample amount was about 100mg, and the elution volume was 100µl. Finally, used Nanodrop 2100 to measure the concentration and purity. Took 1-2 µg of sample and analyzed by 1% agarose gel electrophoresis. From the results, we can get the following conclusions:

- 1. Odorless TCEP can be used instead of strongly odorous mercaptoethanol. Compared with the control group without TCEP and mercaptoethanol, TCEP and hydrophobic ethanol can significantly prevent the browning reaction of the lysate. From the result of electrophoresis, the addition of TCEP and mercaptoethanol can also prevent RNA degradation (compared with the non-added group, the results are not shown). From the analysis of OD values, compared with the addition of 5% mercaptoethanol, adding TCEP (20Mm) as an antioxidant in the lysis buffer resulted in higher RNA production and a better A260/230 ratio, which is closer to 2.0. This is because TCEP has a stronger ability to break protein disulfide bonds which improves lysis ability.
- From the analysis of OD values, R4150 has a high RNA yield, ranging from 12-60µg for different plant leaves at 100mg.
  The purity of RNA extraction is high, with A260/280 ranging from 2.0 to 2.1 and A260/230 ranging from 1.5 to 2.4.



From the result of electrophoresis, without the addition of DNase treatment, DNA contamination cannot be seen on the electrophoresis image, indicating that the DNA filter column can efficiently remove DNA.

- 3. RNA integrity. From the result of electrophoresis, some plant leaves show ideal 18S and 28S bands (1:2 brightness) indicating that RNA has not undergone degradation.
- 4. Some plant leaves (such as paper mulberry, sweet potato, and tomato) exhibit multiple bands due to their unique species characteristics, but it can also be inferred that degradation has not occurred [Figure 3: Excessive RNA loading, insufficient electrophoresis, incomplete staining of large fragments, slightly poor electrophoresis image]
- 5. Magen R4150 is evolved from R4151, using a milder lysis method to reduce damage to some organelles. Therefore, it can be successfully applied to various types of samples, including polyphenol polysaccharide samples. However, due to the mild nature of the lysis buffer, there is a higher risk of column blockage when passing through RNA columns, so the recommended sample size is 50-100mg. 【R4151 is not suitable for polyphenol samples and cannot be used for grapes, tea leaves, or eucalyptus trees, but when processing easy-to-extract samples, the sample amount can reach 200-300mg】